

# Biofilms and antimicrobial resistance: a hidden driver in the one health AMR crisis

Rachna Jain<sup>1\*</sup>, Rupsa Biswas<sup>1</sup>, and Mrigakkhi Ray<sup>1</sup>

<sup>1</sup> CSIR-NEERI, Kolkata Zonal Centre, i-8, Sector-C, EM Bypass, Kolkata-700107, India.

## Abstract

Antimicrobial resistance (AMR) is a grand challenge threatening global health, food systems, and ecosystems. Biofilms—structured microbial communities encased in an extracellular matrix—are a pervasive but underappreciated driver of AMR across the One Health continuum (humans, animals, and the environment). Biofilm physiology elevates antimicrobial tolerance through diffusion barriers, altered microenvironments, stress responses, and persister formation, while promoting horizontal gene transfer (HGT) of resistance determinants. In clinical care, biofilms complicate device-associated infections, chronic wounds, and respiratory disease. In agri-food systems, they colonize farm environments, food processing equipment, and aquaculture infrastructure. In natural and built environments, biofilms act as reservoirs and reactors for resistance genes and antibiotic residues. This review synthesizes current understanding of biofilm-driven AMR across One Health, highlights advance in detection and control, and outlines prioritized policy, surveillance, and research actions to address this hidden driver.

**Keywords:** Antimicrobial Resistance; Biofilms; One Health; Extracellular Polymeric Substances; Persisters; Horizontal Gene Transfer; Diagnostics; Therapeutics; Wastewater; Surveillance

\* Corresponding Author: Dr Rachna Jain, Principal Scientist  
Email: [rachna11587@gmail.com](mailto:rachna11587@gmail.com); [rachana.jain@csir.res.in](mailto:rachana.jain@csir.res.in)

## 1. Introduction: AMR through a One Health lens

Antimicrobial resistance (AMR) emerges from interconnected human, animal, and environmental domains where antimicrobials, microbes, and genes cycle bi-directionally. While policy and surveillance often emphasize planktonic pathogens, most microbes in nature inhabit surface-attached biofilms. AMR is inherently a One Health challenge, arising from the interconnected misuse and dissemination of antimicrobials across humans, animals, food systems, and the environment (1). Biofilm-associated cells display up to 10–1000×-reduced susceptibility to antimicrobials compared with planktonic counterparts, leading to persistent infections and environmental reservoirs of resistance (2-5). The extracellular polymeric substance (EPS) matrix, steep gradients in oxygen and nutrients, and biofilm-specific gene expression jointly remodel physiology, producing phenotypes that evade immune responses and pharmacologic exposures (4-8). Recognizing biofilms as a cross-cutting AMR driver reframes priorities for stewardship, sanitation, and innovation across the One Health continuum.

### Novelty and contribution of the present review

While biofilm-associated antimicrobial tolerance and resistance mechanisms have been extensively examined in clinical microbiology, their role as a unifying driver of antimicrobial resistance across the interconnected human–animal–environment continuum has not been systematically synthesized. The present review advances existing knowledge by: (i) explicitly framing biofilms as a cross-cutting One Health AMR amplifier linking clinical infections, agri-food systems, and environmental resistomes; (ii) integrating environmental engineering, materials science, and microbiology perspectives to evaluate translational anti-biofilm interventions; (iii) contextualizing biofilm-driven AMR within global surveillance frameworks (e.g., GLASS and wastewater-based epidemiology); and (iv) proposing prioritized biofilm-aware policy and infrastructure actions. By positioning biofilms as active ecological reactors of resistance evolution rather than passive microbial aggregates, this review reframes AMR containment strategies across healthcare, agriculture, and environmental governance.

## 2. Review methodology and scope

A narrative-integrative review methodology was adopted to synthesize mechanistic, translational, and policy-relevant evidence on biofilm-associated AMR within a One Health framework. Literature searches were conducted using PubMed, Web of Science, Scopus, and Google Scholar for

publications from 1999 to March 2025. Search strings included combinations of: “biofilm” AND “antimicrobial resistance” OR “antibiotic tolerance” OR “horizontal gene transfer” OR “environmental resistome” OR “wastewater” OR “food processing” OR “medical devices” OR “One Health”.

Priority was given to systematic reviews and meta-analyses, landmark mechanistic studies, environmental surveillance datasets, and WHO/EFSA/CLSI/EUCAST/GLASS policy documents. Inclusion criteria comprised studies that examined biofilm-mediated tolerance, resistance evolution, or resistome dissemination; addressed clinical, agricultural, aquaculture, wastewater, drinking water, or food-processing biofilms; and investigated HGT, co-selection, or persistence dynamics. The scope integrates molecular mechanisms, ecological dynamics, surveillance tools, and governance frameworks to evaluate biofilms as a hidden but critical driver of the global AMR crisis.

## 3. What are biofilms? Structure and function relevant to AMR

### 3.1 Definition and architecture

Biofilms are multicellular assemblies embedded in a self-produced matrix of polysaccharides, proteins, lipids, and extracellular DNA (eDNA) (6,9). The matrix forms a viscoelastic scaffold with water channels that modulate transport, while binding antimicrobials and host factors (6,9,10).

### 3.2 Microenvironments and heterogeneity

Biofilms exhibit microscale gradients in oxygen, pH, redox state, and metabolites that segment the community into subpopulations: rapidly dividing surface layers, slow-growing interior cells, and dormant persisters (7, 11-13). This spatial heterogeneity is central to antimicrobial tolerance because most antibiotics act on active cellular processes.

### 3.3 Developmental program

Biofilm formation proceeds through reversible attachment, irreversible adhesion, microcolony maturation, and dispersal. Quorum-sensing and cyclic-di-GMP signaling regulate matrix production, motility, and detachment, with species- and niche-specific features (14-16).

## **4. How biofilms drive antimicrobial tolerance and resistance**

### **4.1 Matrix-mediated sequestration and diffusion limitation**

The EPS matrix impedes antibiotic penetration and can chemically sequester or inactivate agents (e.g., aminoglycoside chelation by eDNA) (6,10,17).

### **4.2 Physiological adaptation and stress responses**

Biofilm cells upregulate efflux pumps, SOS and stringent responses, oxidative stress defenses, and biofilm-specific regulons that reduce drug susceptibility (8,12,18). Low metabolic rates in interior zones blunt the activity of growth-dependent antibiotics (7,11,13).

### **4.3 Persisters and tolerance reservoirs**

Persister cells are transient, non-heritable phenotypes that survive lethal exposures and reseed communities post-therapy (11,12,19). Their frequency increases under biofilm-associated stresses (nutrient limitation, oxidative stress) (11,19).

### **4.4 Horizontal gene transfer (HGT)**

Dense cell packing and eDNA-rich matrices enhance conjugation, transformation, and transduction. Biofilms facilitate the exchange and maintenance of plasmids, integrons, and transposons encoding resistance (20-23). Sub-inhibitory antibiotic concentrations—common in biofilms—can further select for HGT and mutagenesis.

### **4.5 From tolerance to heritable resistance**

Repeated treatment failure against tolerant biofilms selects for stable resistance mutations and mobile resistome expansion, blurring operational distinctions between tolerance and resistance (12,22,24).

### **4.6 From tolerance to resistance: unresolved questions and controversies**

Despite strong experimental evidence supporting biofilm-mediated antimicrobial tolerance, the quantitative contribution of biofilms to the global AMR burden remains poorly resolved. A central controversy concerns whether tolerance primarily serves as a transient survival phenotype or acts as a direct evolutionary accelerator for heritable resistance. While persisters do not carry resistance mutations, repeated therapeutic failure against tolerant biofilm populations creates strong selection pressure for stable resistance determinants. Sub-inhibitory antibiotic concentrations commonly detected in wastewater biofilms

further promote mutagenesis and HGT. Another unresolved question concerns the representativeness of *in vitro* biofilm models, which may not capture polymicrobial structure, hydrodynamic shear, host immune pressure, and chemical gradients present in chronic infections or wastewater systems.

## **Results: Evidence Synthesis**

## **5. Biofilm-driven AMR across One Health domains**

### **5.1 Human health (clinical and public health)**

#### **5.1.1 Device- and wound-associated infections**

Indwelling medical devices and chronic wounds harbor biofilms from pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida* spp., driving recalcitrant infections and reinfection cycles (3-5,25-28).

#### **5.1.2 Respiratory biofilms**

Chronic lung diseases (e.g., cystic fibrosis, bronchiectasis, COPD) feature polymicrobial biofilms with heightened tolerance; *P. aeruginosa* biofilms exemplify adaptive resistance and frequent HGT as discussed in section 4.4 (3,13,29).

#### **5.1.3 Public health implications**

Biofilm-laden water systems (hospital plumbing, dental unit waterlines) and high-touch surfaces act as reservoirs for healthcare-associated pathogens and resistance genes (30-32).

### **5.2 Animal health and agri-food systems**

#### **5.2.1 Livestock and poultry**

Biofilms colonize watering lines, milking equipment, and housing surfaces; *Salmonella*, *Campylobacter*, and *Staphylococcus* biofilms contribute to on-farm persistence and transmission (33-35).

#### **5.2.2 Aquaculture**

Recirculating systems and nets host biofilms subjected to antibiotic use and metals, promoting co-selection and dissemination of resistance determinants (36-37).

#### **5.2.3 Food processing**

Biofilms on processing lines and drains enable persistence of *Listeria monocytogenes* and other foodborne pathogens despite sanitation (38,39).

#### 5.2.4 Plant interfaces

Plant pathogens like *Xanthomonas* and *Pseudomonas syringae* form biofilms on leaf surfaces and vascular tissues, causing persistent plant diseases. The protective EPS matrix reduces the efficacy of bactericides and fungicides. (40)

### 5.3 Environment and the environmental resistome

#### 5.3.1 Wastewater and receiving waters

Wastewater treatment plants (WWTPs), hospital effluents, and combined sewer overflows are hotspots where antibiotics, biocides, metals, and microorganisms co-mingle in biofilms on pipes and reactors, fostering selection and exchange of resistance genes (41-45). Effluent and sludge can disseminate ARGs to surface waters and soils (42-45).

#### 5.3.2 Built environment microbiomes

Drinking water distribution systems, premise plumbing, and cooling towers harbor diverse biofilms shaped by disinfectant residuals and pipe materials (30, 46-48).

#### 5.3.3 Co-selection by biocides and metals

Environmental exposures to disinfectants and heavy metals can co-select for AMR via shared efflux systems and co-located genes on mobile elements (49-50).

### 5.4 Quantitative context across domains

Tolerance increases of 10–1000-fold are frequently cited, but magnitude varies by species composition, matrix chemistry, hydrodynamic regime, antimicrobial class, and exposure duration. Environmental biofilms often exhibit lower apparent tolerance than clinical biofilms; however, their ecological significance lies in long-term selection, gene exchange, and dissemination rather than acute treatment failure. Thus, clinical tolerance manifests as therapeutic recalcitrance, whereas environmental tolerance functions as a chronic evolutionary incubator of resistance. Figure. 1 depicts the dissemination of biofilms lead AMR formation in different environmental conditions.

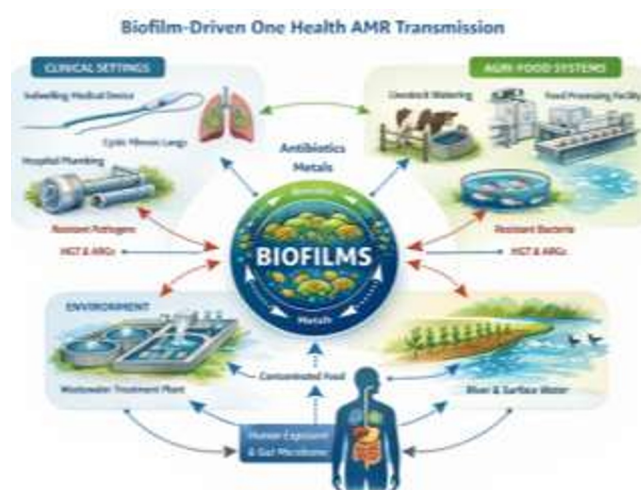


Figure 1. Biofilm-driven One Health AMR transmission network.

## 6. Detection and surveillance

### 6.1 Phenotypic assays

Crystal violet microtiter assays, colony biofilm models, and flow cells quantify biomass and architecture but under-represent polymicrobial, *in situ* complexity (51-53).

### 6.2 Microscopy and imaging

Confocal laser scanning microscopy with fluorescent reporters enables 3D visualization and live/dead mapping; microelectrodes and Raman micro spectroscopy reveal chemical gradients (6,52,54).

### 6.3 Genomics and resistome analytics

Shotgun metagenomics and long-read sequencing profile taxonomic and ARG composition; plasmid-resolved assemblies and Hi-C link ARGs to hosts (43,55,56). qPCR panels quantify sentinel ARGs. Integrating biofilm sampling into GLASS-like surveillance would better capture environmental and device-associated reservoirs (57-59).

### 6.4 Standardization gaps

Lack of harmonized biofilm sampling methods, clinically relevant susceptibility testing for biofilms, and cross-sector data integration remain barriers (53,58,59).

Despite methodological advancements, biofilm-aware AMR surveillance is still constrained by a lack of uniform sampling, a lack of clinically validated biofilm susceptibility tests, the high cost of advanced omics, and lax regulatory requirements. These barriers hinder routine

integration into healthcare and environmental monitoring frameworks.

## 7. Interventions and control strategies

### 7.1 Preventing attachment and matrix formation

Surface engineering (anti-adhesive, zwitterionic, or micro-topographical coatings) and materials releasing nitric oxide or quorum-sensing inhibitors reduce initial colonization (60-63).

### 7.2 Matrix-disrupting adjuvants

Enzymes (DNases, dispersin B), chelators, and mucolytics enhance antibiotic penetration; physical modalities (ultrasound, electrical stimulation, photodynamic therapy) augment disruption, especially in wound care (17-66).

### 7.3 Targeting physiology and persisters

Cyclic-di-GMP modulators, metabolic adjuvants (e.g., sugars to potentiate aminoglycosides), and anti-persister strategies (e.g., ADEP4 activating ClpP) restore antibiotic activity in tolerant subpopulations (12,19,67,68).

### 7.4 Anti-virulence and quorum-sensing interference

QS inhibitors and anti-virulence agents disarm pathogens without strong selective pressure for resistance (15,63,69).

### 7.5 Biologics and alternatives

Bacteriophages and phage-derived endolysins penetrate and disrupt biofilms; combinations with antibiotics or depolymerases show synergy. Probiotics and bacteriocins modulate colonization in food systems and aquaculture (70-74).

### 7.6 Hygiene, sanitation, and stewardship

Rigorous cleaning-in-place, biofilm-aware sanitation schedules, and prudent antimicrobial use across human and veterinary medicine reduce selection pressure and surface colonization (33,35,38,57).

This section approaches differ markedly in evidence strength and translational readiness. Surface engineering and improved sanitation are currently the most mature and widely deployable strategies. Enzymatic matrix disruptors and physical adjuncts show strong preclinical and early clinical promise, particularly in wound care. Phage therapy, quorum-sensing inhibitors, and anti-persister agents remain largely experimental but offer high long-term potential if regulatory and manufacturing hurdles are addressed.

## 8. Cross-domain transmission pathway

Biofilm-associated resistome circulate through interconnected ecological and infrastructural pathways. WWTPs act as convergence nodes where clinical, agricultural, and community-derived antibiotics, metals, and microorganisms co-mingle in dense biofilm reactors. Effluent discharge disseminates ARGs into rivers used for irrigation, facilitating soil and crop colonization. Food crops and aquaculture products then act as secondary exposure routes to the human gut microbiome. Hospital plumbing biofilms and municipal drinking water systems form bidirectional reservoirs for healthcare-associated pathogens. Agricultural runoff introduces manure-derived biofilms into surface waters, while aquaculture facilities recycle biofilm-laden water through recirculating systems. These feedback loops transform biofilms into ecological amplifiers rather than passive reservoirs, linking antimicrobial use in one sector directly to resistance emergence in another.

## 9. Discussion

### 9.1 Biofilms as ecological amplifiers of antimicrobial resistance

The evidence synthesized in this review supports a paradigm shift in AMR governance: biofilms must be recognized not merely as clinical complications but as ecological amplifiers of resistance evolution. Their spatial structure, metabolic heterogeneity, and genetic exchange networks generate evolutionary conditions unmatched by planktonic systems. Current AMR strategies disproportionately focus on antimicrobial stewardship while underestimating infrastructural reservoirs such as wastewater reactors, hospital plumbing, and food-processing drains. Without addressing these persistent ecological reactors, resistance suppression in one domain will continue to be undermined by selection in another. A biofilm-aware, One Health framework therefore demands integration of microbiology, engineering, materials science, public health, and governance.

AMR's ecological amplifiers: biofilms, the data backs with the idea that biofilms are ecological amplifiers of resistance evolution rather than only clinical issues.

Current AMR strategies overemphasize stewardship while underestimating infrastructural reservoirs such as wastewater reactors, hospital plumbing, and food-processing drains. Without addressing these systems, resistance suppression in one domain will be undermined by selection in another.

## 10. Policy, governance, and research priorities

Biofilm-aware AMR control is shaped by policy, governance, and research agendas in addition to technical difficulties and ethical and economic factors.

Responsibility for monitoring environmental resistome remains poorly defined, while infrastructure upgrades and advanced diagnostics pose substantial cost barriers, particularly in low- and middle-income settings. Addressing these gaps requires coordinated regulatory frameworks, sustained investment, and cross-sector accountability.

### Tier 1: Immediate actions (1–3 years)

- Integrate biofilm sampling into wastewater-based AMR surveillance
- Include hospital plumbing and food-processing drains in routine monitoring
- Harmonize environmental ARG panels with GLASS indicators

### Tier 2: Mid-term actions (3–7 years)

- Develop CLSI/EUCAST biofilm susceptibility standards
- Mandate biofilm-aware sanitation protocols in food industries
- Regulate environmental antibiotic discharge thresholds

### Tier 3: Long-term structural reforms (7–15 years)

- Redesign healthcare and water infrastructure using anti-biofilm materials
- Incentivize anti-biofilm medical devices
- Establish One Health biofilm observatories

## 11. Conclusion

Biofilms represent a ubiquitous yet underappreciated driver of antimicrobial resistance across the One Health continuum. Their emergent properties matrix protection, physiological heterogeneity, persister formation, and intensified gene exchange create evolutionary conditions that accelerate resistance emergence while undermining therapeutic and sanitation strategies. Positioning biofilms at the centre of AMR surveillance, infrastructure design, and policy reform offers a transformative opportunity for durable resistance containment. A translational research agenda bridging microbiology, engineering, and

implementation science is essential to outpace this hidden driver of the global AMR crisis. Biofilms as ecological amplifiers of AMR, the evidence supports a shift from viewing biofilms solely as clinical complications to recognizing them as ecological amplifiers of resistance evolution. Current AMR strategies overemphasize stewardship while underestimating infrastructural reservoirs such as wastewater reactors, hospital plumbing, and food-processing drains. Without addressing these systems, resistance suppression in one domain will be undermined by selection in another.

## 12. Research Gaps and Future Directions

1. Translational PK/PD models reflecting biofilm physiology.
2. Standardized biofilm diagnostics for clinical and environmental monitoring.
3. Field trials for anti-biofilm surfaces, phage, and nano-based therapies.
4. Risk assessment linking environmental biofilm indicators to human/animal outcomes.
5. Policy integration: harmonized guidelines on discharge standards, device design, and monitoring.

## References

1. Robinson TP, Bu DP, Carrique-Mas J, et al. *Trans R Soc Trop Med Hyg.* 2016;110(7):377–380.
2. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol.* 2004;2(2):95–108.
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999;284(5418):1318–22.
4. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet.* 2001;358(9276):135–8.
5. Flemming H-C, Wingender J. The biofilm matrix. *Nat Rev Microbiol.* 2010;8(9):623–33.
6. Flemming H-C, Wingender J, Szewzyk U, et al. Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol.* 2016;14(9):563–75.
7. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol.* 2008;6(3):199–210.
8. Bjarnsholt T. The role of bacterial biofilms in chronic infections. *APMIS Suppl.* 2013;(136):1–51.
9. Petrova OE, Sauer K. Escaping the biofilm in more than one way: desorption, detachment or dispersion. *Curr Opin Microbiol.* 2016;30:67–78.

10. Chiang WC, Nilsson M, Jensen PØ, et al. Extracellular DNA shields against aminoglycosides in *Pseudomonas aeruginosa* biofilms. *Pathog Dis*. 2013;69(2):158–64.
11. Lewis K. Persister cells, dormancy and infectious disease. *Nat Rev Microbiol*. 2007;5(1):48–56.
12. Keren I, Kaldalu N, Spoering A, et al. Persister cells and tolerance to antimicrobials. *FEMS Microbiol Lett*. 2004;230(1):13–8.
13. Winstanley C, O'Brien S, Brockhurst MA. *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. *Trends Microbiol*. 2016;24(5):327–37.
14. Römling U, Galperin MY, Gomelsky M. Cyclic di-GP: the first 25 years of a universal bacterial second messenger. *Microbiol Mol Biol Rev*. 2013;77(1):1–52.
15. Hentzer M, Givskov M. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J Clin Invest*. 2003;112(9):1300–7.
16. Nadell CD, Xavier JB, Levin SA, Foster KR. The evolution of quorum sensing in bacterial biofilms. *PLoS Biol*. 2008;6(1):e14.
17. Fleming D, Rumbaugh KP. Approaches to dispersing medical biofilms. *Microorganisms*. 2017;5(2):15.
18. Poole K. Efflux-mediated antimicrobial resistance. *J Antimicrob Chemother*. 2005;56(1):20–51.
19. Conlon BP. Persister cells in biomedicine. *Curr Biol*. 2014;24(14):R654–R657.
20. Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunol Med Microbiol*. 2012;65(2):183–95.
21. Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Appl Environ Microbiol*. 1999;65(8):3710–3.
22. Molin S, Tolker-Nielsen T. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilization of the biofilm structure. *Curr Opin Biotechnol*. 2003;14(3):255–61.
23. Domingues S, Harms K, Fricke WF, et al. Natural transformation facilitates transfer of transposons, integrons and gene cassettes among bacteria. *Nucleic Acids Res*. 2012;40(18):e101.
24. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol*. 2014;12(7):465–78.
25. Donlan RM. Biofilms and device-associated infections. *Emerg Infect Dis*. 2001;7(2):277–81.
26. Arciola CR, Campoccia D, Montanaro L. Implant infections: adhesion, biofilm formation and immune evasion. *Nat Rev Microbiol*. 2018;16(7):397–409.
27. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *J Med Microbiol*. 2015;64(4):323–34.
28. Malone M, Bjarnsholt T, McBain AJ, et al. The prevalence of biofilms in chronic wounds: a systematic review. *Adv Wound Care*. 2017;6(12):403–19.
29. Hauser AR, Jain M, Bar-Meir M, McColley SA. Clinical significance of microbial infection and biofilms in cystic fibrosis. *Clin Microbiol Rev*. 2011;24(1):29–70.
30. Wingender J, Flemming H-C. Biofilms in drinking water and their role as reservoir for pathogens. *Int J Hyg Environ Health*. 2011;214(6):417–23.
31. Walker JT, Bradshaw DJ, Finney M, et al. Microbiological evaluation of dental unit water systems. *J Hosp Infect*. 2000;46(2):148–52.
32. Rickard AH, Gilbert P, High NJ, et al. Bacterial coaggregation in mixed-culture biofilms. *J Appl Microbiol*. 2003;95(5):744–55.
33. Bridier A, Sanchez-Vizueté P, Guilbaud M, et al. Biofilm-associated persistence of food-borne pathogens. *Food Microbiol*. 2015;45(Pt B):167–78.
34. Fagerlund A, Møretro T, Heir E, et al. Cleaning and disinfecting agents can select for antibiotic resistance. *Microbiology*. 2016;162(10):1737–43.
35. EFSA BIOHAZ Panel. Scientific opinion on the role of biofilms in persistent *Listeria monocytogenes* contamination of food processing environments. *EFSA Journal*. 2018;16(7):5305.
36. Defoirdt T, Sorgeloos P, Bossier P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr Opin Microbiol*. 2011;14(3):251–8.
37. Romalde JL, Toranzo AE. Bacterial infections in marine fish: biofilm involvement and control. *Int Microbiol*. 2022;25:541–55.
38. Simões M, Simões LC, Vieira MJ. A review of current and emergent biofilm control strategies. *LWT – Food Sci Technol*. 2010;43(4):573–83.
39. Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ. *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *J Food Prot*. 2014;77(1):150–70.
40. Ramey BE, et al. *Annu Rev Phytopathol*. 2004;42:117–1
41. Rizzo L, Manaia CM, Merlin C, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Sci Total Environ*. 2013;447:345–60.
42. Michael I, Rizzo L, McArdell CS, et al. Urban wastewater treatment plants: advanced treatment

- to reduce residual antibiotics? *Environ Int.* 2013;61:127–51.
43. Ju F, Li B, Ma L, et al. Antibiotic resistance genes and bacterial communities in urban lakes: linkages between ARGs and microbial community at assemble and species levels. *ISME J.* 2016;10(7):164–75.
  44. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ Int.* 2016;86:140–9.
  45. Manaia CM. Assessing the risk of antibiotic resistance transmission from the environment to humans: non-direct proportionality between abundance and risk. *Trends Microbiol.* 2017;25(3):173–81.
  46. Prest EI, Hammes F, van Loosdrecht MCM, Vrouwenvelder JS. Biological stability of drinking water: controlling factors, methods, and challenges. *Front Microbiol.* 2016;7:45.
  47. Proctor CR, Hammes F. Drinking water microbiology—from measurement to management. *Curr Opin Biotechnol.* 2015;33:87–94.
  48. Douterelo I, Boxall JB, Deines P, et al. Methodological approaches for studying the microbial ecology of drinking water distribution systems. *Water Res.* 2014;65:134–56.
  49. Pal C, Asiani K, Arya S, et al. Metal resistance and antibiotic resistance co-select in natural environments. *Nat Commun.* 2017;8:15503.
  50. Wales AD, Davies RH. Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics (Basel).* 2015;4(4):567–604.
  51. O'Toole GA. Microtiter dish biofilm formation assay. *J Vis Exp.* 2011;(47):e2437.
  52. Azeredo J, Azevedo NF, Briandet R, et al. Critical review on biofilm methods. *Crit Rev Microbiol.* 2017;43(3):313–51.
  53. Ceri H, Olson ME, Stremick C, et al. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol.* 1999;37(6):1771–6.
  54. Neu TR, Lawrence JR. Innovative techniques, sensors, and approaches for imaging biofilms at different scales. *Trends Microbiol.* 2015;23(4):233–42.
  55. Su J-Q, An X-L, Li B, et al. Metagenomics of urban sewage identifies an extensively shared antibiotic resistome across human populations. *Nat Commun.* 2017;8:233.
  56. Stalder T, Press MO, Sullivan S, et al. Linking the resistome and plasmidome to the microbiome. *ISME J.* 2019;13(10):2437–46.
  57. World Health Organization. Global Action Plan on Antimicrobial Resistance. Geneva: WHO; 2015.
  58. International standards (CLSI/EUCAST) discussion papers on biofilm susceptibility testing. *CLSI/EUCAST documents*; various years.
  59. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report. Geneva: WHO; 2022.
  60. Epstein AK, Wong T-S, Belisle RA, Boggs EM, Aizenberg J. Liquid-infused structured surfaces with exceptional anti-biofouling performance. *Proc Natl Acad Sci USA.* 2012;109(33):13182–7.
  61. Reddy ST, Swartz MA. A biomimetic model of interstitial flow and its effects on extracellular matrix deposition. *Biophys J.* 2007;93(7):230–41.
  62. Leng C, Sun S, Zhang K, Jiang S. Zwitterionic surfaces for antifouling: mechanisms and applications. *Acta Biomater.* 2016;40:6–16.
  63. Hentzer M, Riedel K, Rasmussen TB, et al. Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology.* 2002;148(Pt 1):87–102.
  64. Tetz GV, Artemenko NK, Tetz VV. Effect of DNase and antibiotics on biofilm characteristics. *Antimicrob Agents Chemother.* 2009;53(3):1204–9.
  65. Qian Z, Sagers RD, Pitt WG. The effect of ultrasonic frequency upon enhanced killing of *Pseudomonas aeruginosa* biofilms. *Ann Biomed Eng.* 1999;27(1):132–8.
  66. Dai T, Huang Y-Y, Hamblin MR. Photodynamic therapy for localized infections—state of the art. *Photodiagnosis Photodyn Ther.* 2009;6(3–4):170–88.
  67. Barraud N, Buson A, Jarolimek W, Rice SA. Mannitol enhances antibiotic sensitivity of *Pseudomonas aeruginosa* biofilms by reversing metabolic states. *PLoS One.* 2013;8(12):e84220.
  68. Conlon BP, Nakayasu ES, Fleck LE, et al. Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature.* 2013;503(7476):365–70.
  69. LaSarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol Mol Biol Rev.* 2013;77(1):73–111.
  70. Abedon ST. Bacteriophage exploitation of bacterial biofilms: phage preference and penetration. *J Bacteriol.* 2011;193(15):3730–6.
  71. Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy. *Future Microbiol.* 2013;8(6):769–83.
  72. Fischetti VA. Bacteriophage endolysins: a novel anti-infective paradigm. *Clin Microbiol Rev.* 2005;18(4):544–61.

73. Defoirdt T. Quorum-sensing systems as targets for antivirulence therapy in *Vibrio* infections. *Int J Mol Sci.* 2018;19(7):2067.
74. Lebeaux D, Ghigo J-M, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance. *Microbiol Mol Biol Rev.* 2014;78(3):510–43